Calcium and Dairy Product Modulation of Lipid Utilization and Energy Expenditure

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Objective: The purpose of this study was to investigate the impact of dietary calcium or dairy product intake on total energy expenditure (TEE), fat oxidation, and thermic effect of a meal (TEM) during a weight loss trial.
Methods and Procedures: The intervention included a prescribed 500-kcal deficit diet in a randomized placebo-controlled calcium or dairy product intervention employing twenty-four 18 to 31-year-old (22.2 ± 3.1 years, mean ± s.d.) overweight women (75.5 ± 9.6 kg). TEM and fat oxidation were measured using respiratory gas exchange after a meal challenge, and TEE was measured by doubly labeled water. Fat mass (FM) and lean mass (fat-free mass (FFM)) were measured by dual-energy X-ray absorptiometry. Subjects were randomized into one of these three intervention groups: (i) placebo (<800 mg/day calcium intake); (ii) 900 mg/day calcium supplement; (iii) three servings of dairy products/day to achieve an additional 900 mg/day.
Results: There were no group effects observed in change in TEE; however, a group effect was observed for fat oxidation after adjusting for FFM ($P = 0.02$). The treatment effect was due to an increase in fat oxidation in the calcium-supplemented group of 1.5 ± 0.6 g/h, $P = 0.02$. Baseline 25-hydroxyvitamin D (25OHD) was positively correlated with TEM ($R = 0.31$, $P = 0.004$), and trended toward a correlation with fat oxidation ($P = 0.06$), independent of group assignment. Finally, the change in log parathyroid hormone (PTH) was positively correlated with the change in trunk FM ($R = 0.27$, $P = 0.03$).
Discussion: These results support that calcium intake increases fat oxidation, but does not change TEE and that adequate vitamin D status may enhance TEM and fat oxidation.

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INTRODUCTION

Despite efforts to reduce the prevalence of obesity, incidence has risen since 1960 and current estimates are that 66% of adults aged ≥20 years are overweight and obese (1). Obesity is a multifactorial disease that involves interactions with genetical and environmental factors, however the increased incidence in recent decades demonstrates that environmental factors play a substantial role in its development.

A variety of epidemiological studies support that calcium or dairy product intake is associated with reduced fat mass (FM) or weight (2,3). Further, dietary calcium and dairy products have been shown to enhance weight or FM loss in several intervention studies (4–6), although calcium supplementation has not been effective in all studies (7). The discrepancy in results suggests that an unidentified factor may be contributing to the impact of calcium or dairy product intake on body FM which, when uncontrolled, leads to variable results across studies.

Although results of studies demonstrate no impact of dietary calcium on total energy expenditure (TEE) (8,9), an increase in fat oxidation is one mechanism that has been proposed to mediate the impact of dietary calcium or dairy products on body FM. In a study by Gunther *et al.* (10), fat oxidation was increased during a 1-year randomized controlled dairy product intake intervention in young women ($n = 26$), but was not increased acutely after a high-dairy product meal. These results suggest that habitual, not acute, intake of dietary calcium or dairy products increases fat oxidation. Although other studies (8) support the association of higher calcium or dairy intake with increased fat oxidation, not all do (9), and thus the issue remains controversial.

Several mechanisms have been proposed by which dietary calcium or vitamin D may regulate fat oxidation, including regulation of parathyroid hormone (PTH, (10)) and the active metabolite of vitamin D, 1,25-dihydroxyvitamin D

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($1,25(OH)_2D$). In addition to maintaining serum calcium homeostasis, PTH and $1,25(OH)_2D$ have a variety of other functions. Serum levels of PTH have been found to be positively associated with changes in FM (11) and fat oxidation (10). PTH levels are also inversely regulated by vitamin D status, as assessed by 25-hydroxyvitamin D (25OHD) (12,13). The impact of calcium or vitamin D status on PTH, may in turn, mediate the systemic effects of these dietary nutrients, but potential relationships with energy metabolism have not been well examined.

The purpose of this study was to determine the impact of dietary calcium or dairy product intake on TEE, fat oxidation, and thermic effect of a meal (TEM) during a randomized controlled weight loss trial. The hypothesis tested was that higher intakes of calcium, either from calcium supplements or dairy products, during an energy-restricted diet would lead to an increase in EE, fat oxidation, and TEM. Further, it was hypothesized that higher PTH would be associated with reduced fat oxidation and that 25OHD would be associated with change in TEM.

METHODS AND PROCEDURES

Subjects

This current investigation was a substudy of a multisite trial completed at four sites (University of Tennessee, Purdue University, Ohio State University, and the University of California-Davis) to investigate the impact of dietary calcium or dairy products in modulating weight loss (6). The parent multisite trial studied 105 otherwise healthy, overweight and obese adults according to the same procedures as described below for this study. Energy metabolism and substrate utilization measures that were completed only at the Purdue University were used in this study and the doubly labeled water analysis was conducted at the University of Wisconsin. Overweight and obese women were solicited through flyers, radio announcements, direct mailings, and information booths located outside residential hall cafeterias. Inclusion criteria included BMI 25–34.9 kg/m^2, 18–35 years of age, consumption of <800 mg total calcium per day (determined by dietary records), and no ~3 kg weight loss in the past 3 months. All the procedures were approved by the Committee on the Use of Human Subjects in Research at the Purdue University and the University of Wisconsin.

Experimental design

This study was designed to determine the impact of dietary calcium or dairy product intake on TEE, fat oxidation, and TEM during a weight loss trial in healthy overweight and obese young adults. Subjects were studied for a 2-week lead-in period to establish their current caloric requirements and provide an opportunity for baseline dietary and physiological assessment, and then randomized into the following intervention groups for 12 weeks: (i) a control diet providing a 500-kcal/day deficit, 0–1 servings of dairy products/day, 500 mg calcium per day, and a daily placebo supplement; (ii) a calcium-supplemented diet identical to the control diet, with the placebo replaced by 900 mg calcium in the form of calcium carbonate; or (iii) a high-dairy diet (placebo supplemented) providing a 500 kcal/day deficit and containing three daily servings of dairy products (milk, cheese and/or yogurt), to bring the total calcium intake from 500 to 1,400 mg/day. The first two arms of the study were conducted in a placebo-controlled, blinded fashion, while the third arm (dairy) was by necessity unblinded. Baseline fasting serum PTH and 25OHD were measured. During the lead-in period and during the week prior to completion of the dietary intervention, TEM and fat oxidation were measured using respiratory gas exchange after a meal challenge, and TEE was measured by doubly labeled water. In addition, FM and lean mass (fat-free mass (FFM)) were measured by dual-energy X-ray absorptiometry at these time points.

Anthropometric measures

During a 2-week lead-in period and weekly, weight was measured using a calibrated digital scale and waist circumference was measured at the level of the umbilicus. Height was measured at baseline using a wall-mounted stadiometer with the subject wearing socks. Body fat was measured at baseline and 12 weeks by dual-energy X-ray absorptiometry (Lunar, Madison, WI). The dual-energy X-ray absorptiometry was calibrated daily by using a phantom to assess drift and followed by a calibration block. If results varied by ~2%, the analysis was repeated. Scans were reanalyzed for trunk and lower body FM using software supplied by the manufacturer (Lunar software, version 8.8). For the trunk (android) region the lower boundary was the pelvis, the upper boundary was a line set at 20% of the distance between the pelvis and base of the neck, and the lateral boundaries were the arm cuts. The upper boundary of the lower body (gynoid) region was 1.5 times the height of the android/trunk region below the pelvis cut line. The lower body region height was equal to two times the height of the trunk region and the lateral boundaries were the outer leg cuts.

Biochemical analysis

At baseline and 12 weeks, blood samples were obtained following a 12-h fast and the serum was stored at −80 °C until analysis. PTH level was determined using a commercial immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra-assay variation for PTH was 4.6% and interassay varitation 7.2%. 25OHD was determined by radioimmunoassay (Immunodiagnostics Systems, Fountain Hills, AZ). The intra-assay variation for 25OHD was 7.5% and interassay variation 9.2%. All hormones, including before and after measurements, were assessed in a batch analysis in duplicate.

Dietary intervention protocol

All subjects were prescribed a 500-kcal/day deficit diet and randomized into one of the three groups: (i) control: 0–1 dairy servings/day, 500 mg total calcium/day, placebo supplement; (ii) calcium supplement: 0–1 servings/day, 900 mg/day calcium supplement, 1,400 mg total calcium/day; and (iii) high dairy: 3 dairy servings/day, 1,400 mg total calcium/day, placebo supplement.

Diets

Three-day diet records were completed during a 2-week lead-in period. Subjects were instructed on how to complete diet records by a trained nutritionist. Diet records were analyzed by Food Processor Plus. The estimate of caloric intake was then refined by calculating energy needs using the World Health Organization equations for calculating basal metabolic rate, and adjusted for activity level to provide an estimate of total daily EE (TDEE). TDEE was calculated as 1.3 × calculated basal metabolic rate using the Harris–Benedict equation for subjects engaged in mild daily activity and 1.5 × basal metabolic rate for those engaged in strenuous daily activity. Discrepancies between the estimated TDEE and baseline caloric intake were resolved, if necessary, by repeat diet records reviewed by the nutritionist.

Based on the estimated TDEE for caloric needs, a diet was prescribed employing food exchanges to provide a caloric deficit of ~500 kcal/day. The diets for each of the intervention groups were designed to provide macronutrient and fiber levels similar to the US consumption average (fat 35% of total kcal, carbohydrates 49%, protein 16%, fiber 8–12 g/day). Subjects were instructed to refrain from consuming nutritional supplements and to maintain their usual caffeine intake. Subjects in the high-dairy group were permitted to include full-fat or low-fat milk, cheese, and yogurt, with the fat accounted for in-exchange lists given to each participant.

Subjects maintained daily food records throughout the 12-week intervention. For compliance assessment and statistical analysis of dietary intake, 3 days of the weekly record, including one weekend day, was analyzed using Food Processor Plus. The daily dietary intakes for the study period were calculated as an average of the intakes for weeks 3, 6, 9, and 12.

Total body EE

Total body EE was assessed by the doubly labeled water technique. Participants visited the clinic two times over the 2-week protocol. Prior to visit 1, participants fasted overnight. Body weight was measured in a hospital gown and a baseline urine specimen was collected. A dose of doubly labeled water weighed to the nearest 0.1 g was administered by mouth, the dose bottle was rinsed with 50 ml of water that was also consumed by the participant. The approximate doses were 1.9 g of 10 atom% ^{18}O water and 0.12 g of 99.9 atom% ^{2}H water per kilogram of estimated total body water. Urine samples were collected at ~1, 2, and 6 h postdose concurrent with the indirect calorimetry for basal resting EE, respiratory quotient (RQ) as well as TEM, fat oxidation and RQ following a low-calcium meal challenge. Subjects voided and were given the doubly labeled water upon arrival. The next urine sample was collected after the 1 h resting metabolic rate (RMR). Subjects then rested quietly for 1 h while the meal challenge was prepared. Another urine sample was collected, the meal was consumed, and TEM begun. The final urine sample was collected at the end of the TEM. The subjects were asked to return 6–9 days after the dose to provide two more samples, with at least 1 h between samples. For all urine samples, total volume was recorded and aliquots were stored at –80 °C. Samples were sent to the University of Wisconsin for analysis.

Isotope abundances were measured in duplicate. Deuterium was measured by online chromium reduction using an H/D device interfaced to a Delta Plus Isotope ratio mass spectrometer (Thermo Electron, Waltham, MA) (14). If the duplicates disagreed by ~5‰ (0.8 ppm), analyses of all specimens from that participant were repeated. The ^{18}O abundances were measured by equilibrating 1 ml of urine with 1 ml of CO_2 gas followed by dynamic isotope ratio analysis on a Delta S isotope ratio mass spectrometer (Thermo Electron, Waltham, MA) (15). If the duplicate differed by ~0.5‰ (10 ppm), analyses of the participants' specimens were repeated. TEE was calculated using the equation of Racette *et al.* (16). If the deuterium to ^{18}O dilution spaces were outside the range of 1.00–1.08, or if the TEE values calculated from each of the two pairs of equilibrated and end period urine enrichments differed by ~10%, isotope analyses were repeated and if agreement was not found, the TEE was not reported. Previous studies have demonstrated a within subject reproducibility for TEE in our hands of ~5% (17).

RMR

Subjects arrived at the semi-darkened, thermoneutral laboratory after a 12-h fast and were asked the time of their last meal and caffeine consumption. Female subjects were asked to report their menstrual cycle day. RMR and RQ were measured by indirect calorimetry (canopy method) using a Sensor Medics V_{max} 29n metabolic cart (Sensor Medics, Anaheim, CA) under standard conditions as prescribed by the company. After voiding, subjects were placed in a supine position on a mechanical bed, and a clear, plastic, ventilated canopy was placed over their head. Subjects were asked to remain as still as possible and breathe normally for 60 min. EE and RQ data were collected and averaged for the last 30 min.

Meal challenge

Total caloric content of the meal challenge for each subject was determined as 50% of the resting EE. The meal consisted of noncalcium-fortified orange juice and a shake containing low-calcium soy protein powder, heavy whipping cream, Swiss Miss Sensation hot chocolate mix, and 10 oz water. Macronutrient content for each meal was constant at 55% carbohydrate, 15% protein, and 30% fat. Ingredient amounts and calcium content for each individual shake were determined using the Food Processor Plus computer program.

TEM

TEM procedures were the same as those described for the RMR. Subjects were placed on the bed and under the hood immediately after consuming the meal challenge. EE and RQ were measured for 4 h.

Calculation of TEM and fat oxidation. TEM was calculated by subtracting the RMR from the 4-h EE average after the meal challenge. To calculate fat oxidation from the measured RQ, estimated nitrogen expired and estimated protein oxidized were determined from the following equations:

$$\text{Estimated nitrogen} = \text{RQ} \times \left(\frac{0.125}{4.05}\right) \times \left(\frac{0.16}{1{,}400}\right)$$

$$N\ (\text{g/min}) = \text{RMR (kcal/d)} \times \left(\frac{0.125}{4.05}\right) \times \left(\frac{0.16}{1{,}440}\right)$$

$$\text{Estimated protein} = \frac{\text{Estimated nitrogen}}{0.16}$$

$$\begin{aligned}\text{Fat oxidation} = {} & (1.689 \times \text{VO}_2\ (\text{l/min})) \\ & - (1.689 \times \text{VCO}_2\ (\text{l/min})) \\ & - (0.324 \times \text{estimated protein}).\end{aligned}$$

Physical activity

Three-day physical activity records were collected from all subjects at baseline and 12 weeks. Briefly, participants were counseled to record activity in 15-min periods throughout the day using an activity code defined by nine categories. The categories range from 1 = lying down, (0.26 kcal/kg/15 min) to 9 = intense work/activity (1.95 kcal/kg/15 min). All activity logs were reviewed and the subject was contacted for clarification as needed. An estimate of 24-h EE was calculated based on the results. Participants were asked to maintain their current activity status and report any changes. No subject reported sizable changes in physical activity during the study period.

Statistical analysis

Prior to statistical analysis, subject adherence to the protocol was assessed based on the *a priori* criteria to establish compliance. For the low-dairy group (low-calcium or high-calcium supplement), compliance was defined as <600-mg calcium in the daily diet from foods, <1 daily serving of dairy in the diet, energy intake within 200 kcal of energy prescription, and return pill counts reflecting utilization of 80–100% of the placebo or calcium supplements provided each week. For the high-dairy group, compliance was defined as >900 mg calcium in the daily diet from foods, ≥3 daily servings of dairy in the daily diet, energy intake within 200 kcal of energy prescription, and return pill counts reflecting utilization of 80–100% of the placebo supplements provided each week. Only subjects who met all compliance criteria for a given week were recorded as compliant for that week. Total study compliance was then defined as meeting weekly compliance for 75% of the weeks.

Weekly compliance required the appropriate intake of kilocalories, dairy servings, supplement pills or placebo, and total calcium. To meet overall compliance, subjects needed to meet all 4 of the above requirements for 9 of the 12 weeks.

Data were then analyzed for statistical significance for compliant subjects. The change in each response variable (i.e., week 12 minus baseline) was fit to a two-way ANOVA model with treatment. When treatment had no significant effects on dependent variables as in the case of 25OHD relationship to 12-week change in TEM, regression analyses was employed to examine relationships independent of treatment group assignment. Intention to treat was analyzed by last-value carried forward method and, although relationships were less strong in some cases, the direction of the change was consistent with the analyses of the compliant subjects.

RESULTS

Of the 36 subjects recruited, only 5 subjects did not complete the study due to time constraints. Of these subjects, 26 were

compliant as defined. The subjects who completed the study were not statistically different from those who did not complete the study in any of the characteristics noted in Table 1. Two of the twenty-six compliant subjects were men and were considered outliers defined by the lean mass of these subjects.

Table 1 Baseline characteristics of subjects (mean ± s.d.)

Variable	Control (*N* = 9)	Calcium (*N* = 6)	Dairy (*N* = 9)
Age (years)	23.3 ± 3.2	22.4 ± 3.7	21.0 ± 2.6
Weight (kg)*	76.8 ± 1.0[a]	68.2 ± 2.9[b]	72.2 + 2.3[ab]
BMI (height/meter^2)	28.8 ± 2.9	27.1 ± 1.5	27.2 ± 1.0
Percent fat mass	43.1 + 4.5	35.2 + 4.3	30.7 + 2.8
Fat mass (kg)*	32.8 ± 5.0[a]	28.8 ± 2.5[b]	30.7 ± 2.8[a]
Lean mass (kg)	38.2 ± 3.2	40.5 ± 4.4	41.7 ± 4.5
Trunk fat mass (kg)*	2.51 ± 7.10[a]	1.82 ± 4.60[b]	2.46 ± 0.34[a]
Lower body fat mass (kg)*	6.22 ± 1.34[a]	4.55 ± 6.00[b]	5.78 ± 0.90[a]
Waist circumference (cm)	83.1 ± 8.8	78.6 ± 3.5	82.6 ± 3.7
25OHD (ng/ml)	20.2 ± 4.8	19.2 + 5.4	20.1 ± 4.5
Parathyroid hormone (ng/ml)	23.6 ± 4.5	31.1 ± 6.3	24.9 ± 4.8
Calcium intake (mg/day)	690 ± 85	592 ± 104	688 ± 85

Values with different letters are significantly different.
25OHD, 25-hydroxyvitamin D.
*Significant main effect of group.

Table 2 Daily dietary intake of subjects during study (mean ± s.d.)

Variable	Control (*N* = 9)	Calcium (*N* = 6)	Dairy (*N* = 9)
Energy (kcal)	1,346 ± 246	1,243 ± 191	1,435 ± 137[†]
Fat (g)	44 ± 16	41 ± 11	39 ± 11
Protein (g)*	56 ± 13[a]	50 ± 8[a]	71 ± 8[b]
Carbohydrate (g)*	176 ± 28[a]	166 ± 28[a]	198 ± 24[b]
Calcium (mg)*	497 ± 58[a]	414 ± 71[a]	1,273 ± 167[b]
Vitamin D (IU)*	48 ± 18[a]	29 ± 22[a]	242 ± 77[b]
Magnesium (mg)*	148 ± 38[a]	134 ± 30[a]	202 ± 28[b]

Values with different letters are significantly different.
*Significant main effect of group in general linear models. [†]Trend toward significance ($0.05 < P < 0.1$) compared to calcium supplemented.

Therefore, analyses were completed with and without these subjects. The baseline characteristics of subjects ($n = 24$) are shown in Table 1. At baseline, the calcium-supplemented group had lower levels of FM than the other two groups, and lower body weight than the control group. Baseline body weight or FM was included in analyses as a covariate to helping controlling for these initial differences.

Dietary intakes by group assignment during the study are shown in Table 2. Both the control and calcium-supplemented groups differed from the dairy product intake group in protein, carbohydrate, dietary calcium, vitamin D, and magnesium, as would be expected with higher dairy product consumption.

TEE and FM

The TEE, TEM, fat oxidation, and RQ at baseline and 12 weeks for each group are shown in Table 3. Group assignment differences were not found significant for change in TEE, even when adjusted for baseline FFM or body weight, change in FFM or body weight, dietary factors (Table 2), or age. In a regression model, there was a trend toward a change in log PTH (partial $P = 0.09$) negatively predicting the change in TEE when age (partial $P = 0.10$) was included in the model (model $P = 0.08$). None of the measures of body composition (baseline or change), dietary factors (Table 2) or group assignment altered the relationship of change in the log PTH with the change in TEE.

In addition, the change in log PTH was positively correlated with the change in trunk FM ($R^2 = 0.27$, $P = 0.03$), but not with lower body FM ($R^2 = 0.11$, $P = 0.17$) or total FM ($R^2 = 0.05$, $P = 0.38$). There was a significant difference (model $P = 0.006$) in change in PTH between the control group (8.14 ± 2.77) and the dairy group (–8.27 ± 3.2, $P = 0.002$). The change in PTH in the calcium-supplemented group (0.86 ± 3.91) did not achieve significance compared with the control group ($P = 0.07$) and was not different from the dairy product intake group ($P = 0.57$). However, the relationship between the change in log PTH with trunk FM was independent of group assignment or any dietary component described in Table 2.

Fat oxidation and TEM

Group assignment was significant in predicting fat oxidation at 12 weeks (Table 3, $P = 0.02$) and change in fat oxidation (Figure 1 and Table 4, $P = 0.02$). Results are similar when baseline or change in FFM, dietary components (Table 2) or baseline or change in total body, trunk, or lower body FM

Table 3 Energy expenditure, TEM, fat oxidation and RQ at baseline and 12 weeks

	Control (*N* = 9)		Calcium supplement (*N* = 6)		Dairy (*N* = 9)	
Weeks	0	12	0	12	0	12
TEE (kcal)	2,438 ± 310	2,297 ± 381	2,470 ± 328	2,454 ± 198	2,323 ± 304	2,297 ± 260
Fat oxidation (g/h)	2.66 ± 1.90	2.03 ± 1.08	1.17 ± 1.27	3.31 ± 1.03[a]	2.41 ± 1.66	1.97 ± 0.61
TEM (kcal/4 h)	39.7 ± 19.5	42.8 ± 9.9	40.6 ± 13.3	47.8 ± 15.8	29.3 ± 14.0	45.9 ± 12.5
RQ	0.90 ± 0.07	0.91 ± 0.04	0.95 ± 0.06	0.87 ± 0.03[a]	0.89 ± 0.07	0.91 ± 0.03

RQ, respiratory quotient; TEE, total energy expenditure; TEM, thermic effect of a meal.
[a]Significantly different from 0 week of the same group assignment.

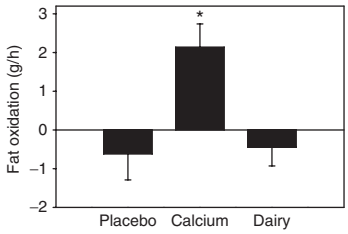

Figure 1 Change in fat oxidation higher in calcium-supplemented group. Fat oxidation after a meal challenge was determined at baseline and 12 weeks of the intervention. After an resting metabolic rate, a meal challenge was given with a total caloric content of the meal for each subject determined as 50% of the resting energy expenditure. The meal consisted of a shake containing low-calcium and macronutrient content for each meal was constant at 55% carbohydrate, 15% protein, and 30% fat. There was a main effect of group assignment (model $P = 0.02$) and asterisk indicates significant difference ($P < 0.05$) in change in fat oxidation in the calcium-supplemented group compared with either the placebo or dairy assignment groups.

Table 4 Relationship between group assignment and changes in TEE, fat oxidation, TEM, and RQ following meal challenge (least square means + s.e. with baseline lean mass included in model)

Changes	Control	Calcium	Dairy	Partial
TEE (kcal)	–131.6 + 81.8	15.59 + 102.6	–56.5 + 84.7	NS
Fat oxidation (g/h)	–0.62 + 0.67[a]	2.14 + 0.60[b*]	–0.44 + 0.49[a]	0.02
TEM (kcal/4 h)	3.11 + 7.84	7.48 + 6.26	16.33 + 6.47*	NS
RQ	0.004 + 0.033[a]	–0.022 + 0.041[b*]	0.053 + 0.03[a]	0.03

Values with different letters are significantly different between groups.
NS, nonsignificant; RQ, respiratory quotient; TEE, total energy expenditure; TEM, thermic effect of a meal.
*Significantly different from 0.

were included in the model. The results demonstrate that an increased fat oxidation in the calcium-supplemented group, but not in the dairy intake group, explains the group assignment effect (Tables 3 and 4).

Finally, there are no differences between any groups in 12-week values of serum 25OHD (control, 20.9 ± 1.5; calcium, 18.0 ± 1.8; dairy, 19.2 ± 1.5) or differences from baseline to 12-weeks serum 25OHD. Baseline 25OHD did not correlate with PTH at baseline ($P = 0.97$) or 12 weeks ($P = 0.11$) even with age in the model ($P = 0.30$). However, baseline, but not 12 week, 25OHD positively predicted the 12-week change in TEM ($R^2 = 0.32$, $P = 0.004$; Figure 2). Further, the mean 25OHD during the study (baseline and 12 weeks) positively predicted the change in TEM ($R^2 = 0.17$, $P = 0.04$). There were no significant differences when each group was analyzed separately for 12-week 25OHD to predict 12-week change in TEM, indicating that the increased vitamin D intake in the dairy group did not contribute significantly to this result. Results were similar when group assignment, age, fat oxidation or PTH at baseline, or differences in fat oxidation, dietary factors (Table 2) or PTH were included in the model.

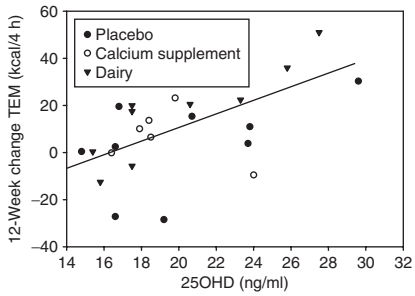

Figure 2 Serum 25-hydroxyvitamin D (25OHD) positively correlates with the 12-week change in thermic effect of a meal (TEM). TEM after a meal challenge was determined at baseline and 12 weeks of the intervention. After an resting metabolic rate, a meal challenge was given with a total caloric content of the meal for each subject determined as 50% of the 24-h resting energy expenditure. The meal consisted of a shake containing low-calcium and macronutrient content for each meal was constant at 55% carbohydrate, 15% protein, and 30% fat. There was no main effect of group assignment, but baseline 25OHD levels predicted the 12-week change in TEM (model $P = 0.02$) independent of group assignment.

DISCUSSION

The results of this study suggest that calcium intake, but not dairy intake, increases fat oxidation during a weight loss trial, and that vitamin D status is associated with energy expended from a meal, or TEM, independent of group assignment. In addition, in this intentional weight loss trial, there is a trend toward an association between a decrease in PTH and increases in TEE and trunk FM, independent of group assignment.

It is difficult to explain why the group consuming dairy products, with similar calcium intakes as the calcium-supplemented group, did not increase fat oxidation whereas the lipid oxidation increased in the calcium-supplemented group compared with the control group. The calcium-supplemented group weighed less and had less FM at baseline. To test whether baseline body composition influenced the change in fat oxidation response, baseline or change in FM, trunk FM or lower body FM were included in the model. None of these body composition measures influenced the results. In addition, neither baseline nor change in 25OHD or log PTH significantly influenced the results. Although this current study suggests that another component of dairy products may prevent the calcium-induced increase in fat oxidation, the small group size and differences in baseline body composition support that this interaction needs further investigation.

A dietary calcium supplement stimulating an increase in fat oxidation is consistent with several other studies (8,10,18,19), but not with all (9). Melanson *et al.* measured 24-h fat oxidation in humans using whole-room indirect calorimetry (8). Healthy, normal-weight, moderately active adult subjects ($n = 24$, 20–45 years old) were housed in a metabolic chamber one time for 24 h. During this time, TDEE and macronutrient oxidation were measured. Habitual calcium intake was estimated from 4-day food diaries, and acute (24 h) calcium was measured from food intake during the time in the chamber. Fat oxidation was positively and significantly correlated with acute total calcium intake ($R = 0.38$, $P = 0.03$) and dairy

calcium intake (R = 0.35, P = 0.04); RQ was negatively and significantly correlated with acute total calcium (R = –0.36, P = 0.04). Habitual intake from dairy or all dietary calcium was not correlated with fat oxidation or RQ. In contrast to other studies, total calcium was a stronger predictor of 24-h fat oxidation than dairy calcium intake. Several studies support that calcium intake from dairy products increases fat oxidation, which is consistent with this study in the calcium-supplemented group, but inconsistent with no change in the dairy product intake intervention group. For example, in the study by Gunther *et al.*, fat oxidation increased in young women after an increase in calcium intake through increased consumption of dairy products for 1 year (10). A significant decrease was found in RQ and increase in fat oxidation (P < 0.05) in response to acute intake of a high-dairy and high-calcium meals in obese men and women (19). Further, there was a higher TEM after the high-calcium intervention with a low-calcium meal challenge, suggesting a metabolic change in TEM mechanisms (10). On the other hand, in a diet intervention of shorter duration, subjects (n = 18) consumed either a low- (500 mg/day) or high- (1,400 mg/day) calcium diet for 7 days in a randomized crossover design (9). In this case changes in resting EE and substrate oxidation were not affected by increased calcium intake.

This study shows a relationship between a decrease in PTH with an increase in both TEE and trunk FM. Fasting serum PTH levels positively correlated with body FM at baseline (n = 155), and change in serum PTH predicted the change in body FM over 1 year in a cohort of normal-weight young women (12). PTH positively correlates with BMI and body FM in a study of 302 adults of mixed race, both obese and nonobese (20). Studies also show that serum PTH levels are higher in obese than in nonobese young adults (21), and hyperparathyroid postmenopausal women have a greater FM with more trunk pattern of fat distribution than age-matched controls (22). Finally, after a year long dairy product intervention in young women, the change in PTH correlated with the change in fat oxidation, independent of control or dairy product intervention group assignment (23), suggesting another factor also regulates fasting levels of PTH and also that PTH may regulate energy metabolism. Thus, taken together, there is substantial evidence, albeit primarily associative, that serum PTH levels modulate FM.

Serum levels of 25OHD, a marker for vitamin D status, are inversely correlated with fasting levels of serum PTH (12,13). It is intriguing that although PTH regulates renal conversion of 25OHD to $1,25(OH)_2D$, in our studies $1,25(OH)_2D$ is positively related to 25OHD, and $1,25(OH)_2D$ shows no relationship with fasting serum PTH levels (24). Although there were no relationships between the serum levels of 25OHD and PTH in this study, the small sample size may have limited the power to detect this relationship. Overweight individuals have lower vitamin D status (20,21,25), and this is generally explained by the hypothesis that vitamin D is sequestered in adipose stores. This association was not noted in this study, suggesting an altered regulatory system for the relationship between PTH and vitamin D status in a weight loss trial compared with other studies of normal-weight women (24). Alternatively, studies suggest that serum levels <32 ng/ml define vitamin D deficiency (26). All the subjects in this study were deficient according to these standards, thus, the narrow range of vitamin D values may have limited the ability to detect a relationship with FM.

The discrepancies between the studies investigating the impact of calcium or dairy products on fat oxidation or body FM suggest that the effect, if it exists, may be multifactorial. Several other dairy product components are hypothesized to contribute to the impact of calcium on energy metabolism, including amino acids and other bioactive components (2,27). This study suggests that vitamin D status may play a role in modulating energy metabolism. One potential hypothesis is that both adequate calcium and vitamin D are required to modulate changes in energy balance or body composition. Further, recent studies suggest that higher levels of vitamin D status, achieved from higher intakes than currently recommended, are needed to suppress serum PTH maximally (28,29). Thus, higher vitamin D intakes than currently recommended may be needed to achieve the optimal health benefits of vitamin D, particularly if levels of serum PTH play a role. Much of the US population would be vitamin D deficient by the new definition of adequacy. It is interesting to consider that higher vitamin D status may promote an increase in EE when combined with other dietary or lifestyle factors. Alternatively, higher 25OHD levels, perhaps above the current definition of adequacy, are required for factors such as calcium to impact overall body EE. This potential effect of vitamin D on modulating energy balance requires further investigation.

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DISCLOSURE

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